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CHARACTERISING THE CHEMICAL TYPICITY OF REGIONAL CABERNET SAUVIGNON WINES

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Abstract

Aim: To define the uniqueness of Australian Cabernet Sauvignon wines by evaluation of the chemical composition (volatile aroma and non-volatile constituents) that may drive regional typicity, and to correlate this with comprehensive sensory analysis data to identify the most important compounds driving relevant sensory attributes.

Methods and Results: A range of specialised analytical methods have been optimised to quantify more than 70 volatile aroma compounds in Cabernet Sauvignon wine. These methods examine a diverse array of metabolites that originate from the grape, fermentation, maturation and oak maturation. Examination of a variety of non-volatile compounds such as tannins, basic chemistry and non-volatile secondary metabolites were also undertaken. These analytes were quantified in 2015 commercial Cabernet Sauvignon wines (n = 52) originating from Coonawarra, Margaret River, Yarra Valley and Bordeaux. Multivariate statistical analysis of chemical datasets and sensory ratings obtained by a trained descriptive analysis panel identified compounds driving aroma attributes that distinguished wines from the different regions. Some compounds, such as dimethyl sulfide, which arises from a grape amino acid and is described as 'black currant or olive' at low concentration and 'canned vegetables' at high concentration, were not statistically different amongst regions. In contrast, compounds such as 1,4-cineole ('mint' and 'bay leaf' aroma), 3-isobutyl-2-methoxypyrazine ('green capsicum' aroma) and 4-ethylphenol ('earthy' and 'band-aid' aroma) were able to differentiate the wines.

Conclusions: For the first time, this work has revealed various wine chemical constituents, both volatile and non-volatile, that have been linked with results from comprehensive sensory analysis to determine the important drivers of regional typicity of Australian Cabernet Sauvignon wines. Identifying these candidates will lead us to the next step of identifying which viticultural and/or winemaking practices can influence these compounds to meet target styles for wines of provenance.

Significance and Impact of the Study: Identifying the chemical markers that characterise Cabernet Sauvignon regional typicity will lead Australian producers one step closer to having the tools to preserve the 'uniqueness' of their regional wines. A greater understanding of chemical drivers of wine sensory traits will keep the industry at the forefront of the field internationally and will provide producers with knowledge that can be used for promoting their wines and enhancing sales.

Keywords: Regional typicity, chemical markers, wine sensory traits, Cabernet Sauvignon

Introduction

Cabernet Sauvignon is a popular grape variety grown in almost all wine producing regions around the world. Originating in Bordeaux in the 1700s (Anderson *et al*., 2013), it is an important variety to Australia where it represents approximately 18% of Australia's vineyard area (Wine Australia, 2020). Learning more about the influences of terroir to understand what drives the regional distinctiveness of this important varietal is imperative for helping maintain Australia's reputation as a fine wine producing and exporting nation.

It is well known that aroma and flavour are responsible for a consumer's enjoyment of wine (Ristic *et al*., 2019), and with over a thousand volatile compounds having been identified in wine to date, the complexity of this matrix becomes evident. Not all of these compounds are present in any one wine, and a much smaller number of them play a vital role in wine flavour (Ferreira *et al*., 2009). These compounds originate from various sources including: the grape berry itself either directly or via precursors (including varietal-specific compounds); alcoholic or malolactic fermentation; oak wood contact; and oxidation and ageing, plus exogenous sources. In previous studies, sixty-nine (Tao *et al*., 2008) and seventy-four (Gürbüz *et al*., 2006) volatile compounds were identified in Cabernet Sauvignon wines. These mainly consisted of higher alcohols, acetates, fatty acids and ethyl esters and to a lesser extent terpenes, a norisoprenoid, carbonyls and phenols.

Varietal-specific compounds originate in the grape berry: a classic example is the methoxypyrazines (MPs), which can impart characteristic aromas of 'green capsicum', 'asparagus' and 'green beans'. These characters are well known for their sensory contribution to wines produced from Cabernet Sauvignon and Sauvignon Blanc (Robinson *et al*., 2014). If present at low ng/L concentrations, MPs are considered desirable and characteristic of the grape cultivar, but at higher concentrations they become unpleasant. 3-Isobutyl-2-methoxypyrazine (IBMP) is the most important of the MPs and is thought to originate from enzymatic methylation of hydroxypyrazines, (Dunlevy *et al*., 2013; Guillaumie *et al*., 2013). A range of factors, including climate, grape maturity, light exposure, various viticultural management practices, and crop yield can affect IBMP concentration. In addition, MPs are easily extracted into must and increase during fermentation on skins (Sidhu *et al*., 2015). They are among the very few wine aroma compounds where the concentration can be predicted from berry homogenates (Ryona *et al*., 2010).

Varietal thiols, another group of varietal character impact compounds, contribute characteristic wine aromas of 'tropical fruit', 'passionfruit', 'citrus', 'grapefruit' and 'box hedge'. Having extremely low odour detection thresholds (low ng/L range), the three most common varietal thiols are 3-sulfanylhexan-1-ol (3-SH), 3-sulfanylhexyl acetate (3-SHA) and 4-methyl-4-sulfanylpentan-2-one (4-MSP) (Roland *et al*., 2011). Their importance in Sauvignon Blanc is well known (Roland *et al*., 2011) and recently their significance in Chardonnay wine has been reported (Capone *et al*., 2018). These compounds were identified in Bordeaux red wines over two decades ago (Bouchilloux *et al*., 1998), but minimal data has become available since then. This is most likely attributable to the challenges associated with developing adequate methods to measure these reactive compounds at ultratrace concentrations (Capone *et al*., 2015). Varietal thiols originate from grape berry-derived odourless cysteinylated and glutathionylated precursors and are released enzymatically during fermentation. As such, there can be significant yeast strain effects, and ripening and post-harvest conditions during winemaking can affect the concentration of thiol precursors (Capone *et al*., 2012c; Chen *et al*., 2019).

1,8-Cineole, commonly known as eucalyptol, is another relevant compound that has an aroma described as 'fresh', 'cool', 'minty' and 'camphoraceous'. Elevated levels of 1,8-cineole in wine may be attributable to matter other than grapes (MOG), in particular material from surrounding *Eucalyptus* trees grown in close proximity to grapevines (Capone *et al*. 2012b). From a large survey of commercial Australian red wines (n = 150) including 45 Cabernet Sauvignon wines, it was shown that 40% of the red wines contained concentrations of 1,8-cineole above its aroma detection threshold and that the concentration increased during fermentation with skin contact (Capone *et al*., 2011). Furthermore, studies showed that once present in wine, 1,8-cineole is extremely stable (Capone *et al*., 2012b). Sensory evaluations of the isomeric compound 1,4-cineole revealed descriptors of 'hay' and 'dried herbs', both independently and in the presence of 1,8-cineole. Quantitative analysis revealed higher concentration of 1,4-cineole in Margaret River Cabernet Sauvignons compared with Barossa Valley, McLaren Vale, and to a smaller degree, Coonawarra Cabernet Sauvignon wines, suggesting this aroma compound could assist with characterising terroir (Antalick *et al*., 2015). Interestingly 1,4-cineole was not present in any of the samples above its aroma detection threshold of 0.6 µg/L (Antalick *et al*., 2015).

Oak volatiles, of which several hundred have been identified in wine (Pollnitz *et al*., 2004), are related to terroir through human intervention, particularly in the winery. These compounds may originate by extraction directly from oak wood, interactions between wood constituents and wine constituents, or through chemical or biological reactions of wood constituents. Only a few of these oak volatiles are thought to be important for wine aroma, with the most noteworthy including *cis*- and *trans*-oak lactone, guaiacol, 4-ethylphenol, 4-methylguaiacol, 4-ethylguaiacol, eugenol and vanillin (Pollnitz *et al*., 2004). The isomers of oak lactone, arguably the most significant of the oak volatiles, exhibit aromas of 'coconut', 'vanilla', 'woody', 'citrus' and 'red berry' for the *cis*-isomer and 'coconut' and 'celery' for the *trans*-counterpart (Gunther *et al*., 1986; Wilkinson *et al*., 2004). Oak used in commercial winemaking can originate from different sources, the most popular being from France and America, adding another layer of terroir impacting on the oak volatile concentrations. For example, it has been demonstrated that 10:1 and 1:1 ratios of *cis*:*trans* oak lactone are indicative of American and French oak, respectively (Waterhouse *et al*., 1994). 4-Ethylphenol (4-EP) and 4-ethylguaiacol (4-EG) are not so desirable and result from microbial spoilage (*Brettanomyces bruxellensis*). This slow growing yeast can cover a barrel and reside in between staves and reproduce when the barrels are refilled with wine. In addition, Brettanomyces can be present on the grapes, in soil and water, and in the winery environment, and could therefore also be considered as being related to terroir (Rayne *et al*., 2007). 4-EP and 4-EG have aromas that can be described as 'leather', 'horse stable', 'band aid' and 'spice, clove', with a ratio of 8:1 of 4EP:4-EG being indicative of *Brettanomyces* spoilage (Chatonnet *et al*., 1995).

Non-volatile compounds are another class of components present in wine that are important to taste and mouthfeel properties. These include things like tannin, polysaccharides, polyols, and organic acids. Only a few of the compound groups are considered here. With a multitude of compounds being present in any one wine, determining the most important ones responsible for characteristic sensory attributes is undoubtedly a challenge. Chemometric methods for analysis of the different datasets are crucial, and partial least squares regression (PLSR) has been adopted as a technique of choice for linking the chemical and the sensory data (Lee *et al*., 2006). This statistical approach enables the determination of which compound(s) are related to particular sensory attribute(s), and helps to identify the basis for any differentiation of wines from various regions of origin. PLSR was thus applied to a suite of chemical data originating from various sources in 52 Commercial Cabernet Sauvignon wines from four different wine producing regions, along with the data obtained from the sensory descriptive analysis (DA) of the same wines. Preliminary results are presented and discussed in relation to differentiating the regions under investigation.

Material and Methods

Fifty-two commercial 2015 vintage Cabernet Sauvignon wines from Coonawarra (n = 24), Margaret River (n = 10), Yarra Valley (n = 13) and Bordeaux (n = 5) were analysed for a range of compound groups. SIDA-SPME-GCMS methods were optimised for the quantification of C_6 compounds (Capone *et al*., 2012a), 1,4- and 1,8-cineole (Antalick *et al*., 2015; Capone *et al*., 2011), DMS (Segurel *et al*., 2005) and a range of fermentation derived volatiles (Wang *et al*., 2016). Oak volatiles were measured utilising liquid injection based on the published method (Pollnitz *et al*., 2000). IBMP was quantified utilising a SIDA-SPME-GC-MS/MS method adapted from Dunlevy *et al*. (2013). Varietal thiols were quantified by HPLC-MS/MS (Capone *et al*., 2015). Tannin and total phenolics were determined by AWRI Commercial Services and non-volatile profiling of secondary metabolites was undertaken through Metabolomics Australia. Basic chemical parameters were analysed as described in Ranaweera *et al*. (2020). All wines underwent sensory profiling using descriptive analysis (DA) as described in Souza Gonzaga *et al*. (2020).

Results and Discussion

Concentrations of a selection of volatile compounds that originate from different sources for the 52 Cabernet Sauvignon wines from four regions are presented in Figure 1. Varietal thiol 3-MH was found at similar concentrations in the Bordeaux and Coonawarra wines, with approximately 250-350 ng/L (Figure 1a). Significantly higher concentrations were measured for Margaret River and Yarra Valley wines, at approximately 280-400 ng/L (Figure 1a). The concentration of 3-MH in all wines was well above the aroma detection of 60 ng/L (determined in a hydroalcoholic solution) (Roland *et al*., 2011), meaning that 3-MH could contribute to the 'berry jam', 'earthy', 'plum' and 'soy' aroma of the wines, as recently reported by Garrido-Bañuelos *et al*. (2020).

The results for 1,4-cineole (Figure 1b) were in reasonable agreement with the previous findings that highlighted 1,4-cineole as being characteristic of Margaret River Cabernet Sauvignon wines. Furthermore, a broader distinction between Bordeaux and the Australian regions existed on the basis of 1,4-cineole concentration (significant differences among the means, $\alpha = 0.05$), but there was no statistical difference between Yarra Valley and Coonawarra wines. The concentrations of 1,4-cineole shown in Figure 1bwere below the aroma detection of this compound, which again was in agreement with the previous study (Antalick *et al*., 2015). In contrast to 1,4-cineole, whose origins are not yet defined, 1,8-cineole has been previously found to be increased by aerial transfer of volatiles from *Eucalyptus* trees to the grapes when vines are planted in the vicinity of the trees, and especially by the presence of MOG (particularly Eucalypt material) ending up in a ferment. Although the concentrations of 1,8-cineole were quite variable, mean values were < 5 µg/L, and there were no significant differences amongst the Australian regions (Figure 1b). Bordeaux wines were found to have < 1 µg/L, but the mean value was only significantly different to that for Coonawarra.

The data for IBMP, an important varietal compound for Cabernet Sauvignon, is shown in Figure 1c. The concentration of IBMP was mostly below the aroma detection threshold of this compound in red wine (10 ng/L (Kosteridis *et al*., 1998)). At these low concentrations, it would be expected that IBMP would not impart any undesirable green characters to the wines. There were no statistically significant differences between the means of IBMP for the Australian regions, whereas Bordeaux was significantly lower than the amounts determined for Coonawarra and Margaret River. This may be attributable to the Bordeaux wines having a lower relative amount of Cabernet Sauvignon in the blend (minimum of 60% vs minimum of 85% for the Australian wines).

The data obtained for *cis*- and *trans*-oak lactone, 4-ethyl phenol and 4-methylguaucol can be seen in Figure 1d. There was no significant difference amongst the regions for *trans*-oak lactone but the *cis*-oak lactone concentration did vary. Interestingly, the ratio of *cis*-oak lactone:*trans*-oak lactone was around 1:1, which is indicative of the predominant use of French oak. Only a few samples had slightly higher ratios, which could infer there was some percentage of American oak being used. Perhaps unsurprisingly, both 4-ethylphenol and 4-ethylguaiacol were significantly higher in Bordeaux wines compared with the Australian wines. These compounds are usually present in higher concentrations as a result of microbiological activity of *Brettanomyces spp* during oak maturation (if the impact of smoke taint can be ruled out). The characteristic ratio of 4-EP:4-EG that is symptomatic of *Brettanomyces* spoilage (Chatonnet *et al*., 1995) appeared to be the reason for the presence of these volatile phenols in the Bordeaux wines (i.e., approximately 8:1 in most of these cases). Notably, one of the Bordeaux samples had 4-EP at up to 378 µg/L, which would undoubtedly have imparted an evident 'band-aid' or 'barnyard' aroma in that wine.

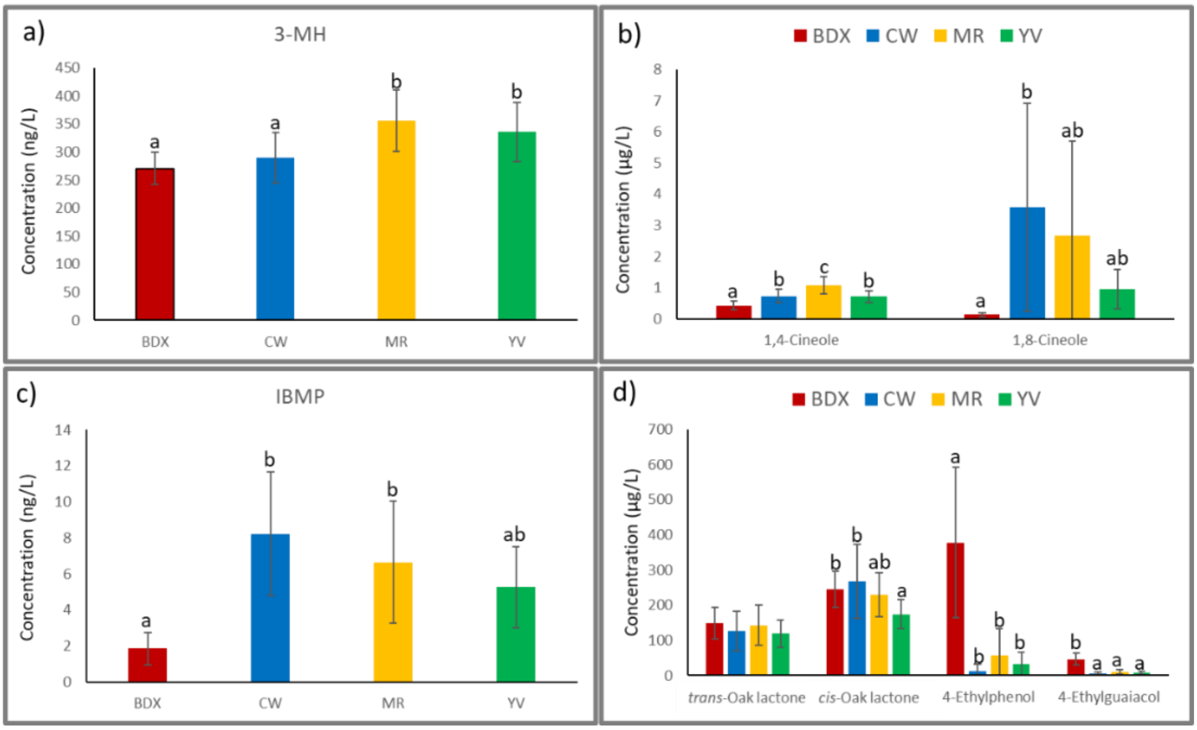

Figure 1: Concentration of a) 3-mercaptohexan-1-ol (3-MH), b) 1,4- & 1,8-cineole, c) 3-isobutyl-2-methoxypyrazine (IBMP), and d) *cis*- and *trans*-oak lactone, 4-ethylphenol and 4-ethylguaiacol in Cabernet Sauvignon from Bordeaux (BDX) (n = 5), Coonawarra (CW) (n = 24), Margaret River (MR) (n = 10) and Yarra Valley (YV) (n = 13). Error bars indicate the standard deviation and different letters above the error bars denote significant differences among the means according to Tukey's post-hoc test (α = 0.05).

To examine any relationships between the regions studied and to determine whether chemical components are able to explain particular sensory attributes, the chemical data generated so far (including non-volatiles, data not shown) was analysed against the sensory DA data using PLSR (Figure 2). With an optimum number of two factors for the preliminary model, the variation of the sensory data was not explained to a high degree (16%) but it gave the first insight into potential regional differences. This will likely be significantly improved once all chemical data is available. From the scores plot, partial separation of the regions was achieved although there is some overlap with certain wines (Figure 2a), which may be expected on the basis of winemaking and style. The Bordeaux wines were clearly separated from the other Australian regions, a large cluster from Coonawarra was separated from Yarra Valley wines, whereas Margaret River seemed more closely related to wines from both Coonawarra and Yarra Valley. Figure 2b indicates that the chemical constituents 4-ethylphenol and 4-ethylguaiacol were driving the separation of the Bordeaux wines, and these compounds were closely related to 'earthy' and 'yeasty' aromas. This relationship could be expected based on the chemical data presented earlier (Figure 1d). A cluster of some of the Coonawarra wines was related to 'minty', 'liquorice', 'dark fruit' and 'spice', with these sensory traits being associated with hexanol, 1,8-cineole, ethyl propanoate and ethyl butyrate, and a range of other compounds to a lesser extent. A cluster of Yarra Valley wines was related to 'red fruit' and 'jammy' characters, and associated with 1-octanol, 2-octanone and DMS. A cluster of wines from Margaret River were characterised by 'grassy', 'eucalypt', 'savoury' and 'chocolate' aromas and were associated with ethyl decanoate, butan-1-ol, (*Z*)-3-hexen-1-ol and ethyl-3-methyl butanol.

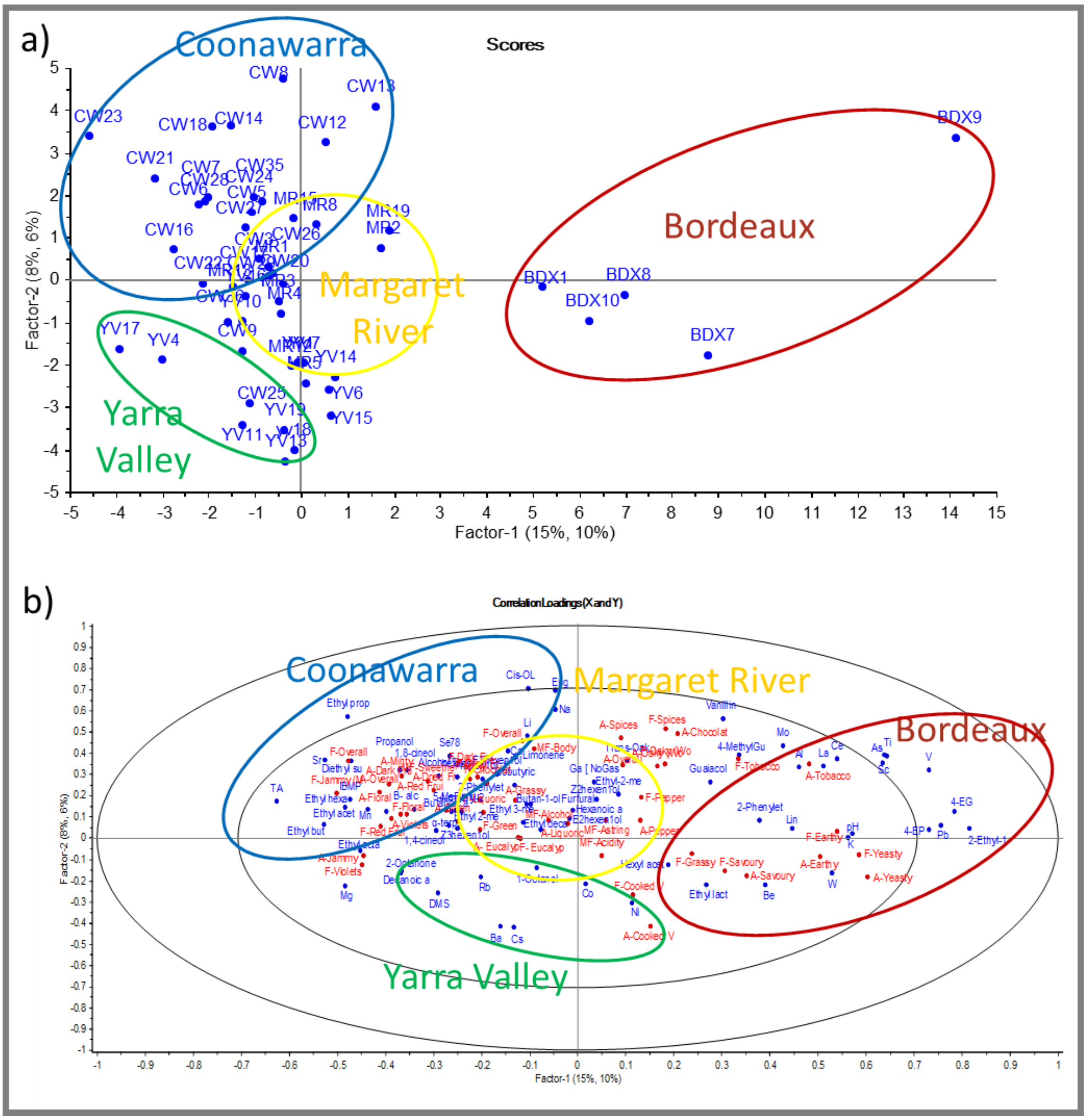

Figure 2: Plot of scores and loadings from PLSR (two factor model) using significant ($P < 0.1$) chemical data (X-variables) and sensory attributes (Y-variables) for the commercial Cabernet Sauvignon wines (n = 52). For the sensory attributes, A = aroma, F = Flavour and MF = Mouthfeel.

In addition to the quantitation of volatiles, elemental analysis data obtained by ICP-MS showed similar trends to those previously reported by (Ranaweera *et al*., 2020). It appeared that separation of Bordeaux wines was driven by the elements K, Sc, Ti, V, As, La, W and Pb, whereas Coonawarra seemed to be higher in Se, Li, Ca and Sr, Margaret River in Co, and Yarra Valley in Mg, Ba, Cs and Rb. Previous research by others has also revealed Sr to be a good marker for authenticating wine (Ranaweera *et al*., 2020).

Conclusions

This work has explored various volatile and non-volatile wine chemical constituents that have been related with sensory descriptive analysis to determine the significant drivers of typicity of regional Cabernet Sauvignon wines. Preliminary data provided examples such as 4-ethylphenol and 4-ethylguaiacol being linked with 'earthy' and 'yeasty' aromas and driving the separation of the Bordeaux wines from the others. Additionally, 1,4-cineole was successful at distinguishing the Australian regions, particularly Margaret River. The varietal compound 3-MH was found to be significantly different in the Bordeaux and Coonawarra wines compared with Margaret River and Yarra Valley, with the amounts being above the aroma detection threshold of this compound. Interestingly, the other varietal compound IBMP, which is an impact compound in Cabernet Sauvignon wines, was lowest in Bordeaux wines. Finalising the remaining chemical data and identifying candidate compounds that differentiate the regions will lead to the next stage, being the of identification of viticultural and/or winemaking practices that can influence wine chemical profiles so that target styles can be met while preserving or enhancing the terroir of the regions.

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